REMARKS

Support for the amendments to claims 9, 10, 15 and 17 can be found, for example, in the originally filed claims and in the Specification at page 5, paragraph 12, through page 7, paragraph 16, and page 15, paragraph 49. Support for new claims 27 and 28 can be found, for example, in the originally filed claims and in the Specification at page 10, paragraph 34.

Restriction requirement

Applicants affirm the election of Group I, corresponding to original claims 1-17. Claims 1-8 and 18-26 have been cancelled without prejudice or disclaimer. Applicants reserve the right to prosecute non-elected subject matter in subsequent divisional applications.

Indefiniteness rejection under 35 U.S.C. §112, second paragraph

Claim 10 was rejected under 35 U.S.C. §112, second paragraph, for alleged indefiniteness. The claim has been revised to clarify the recitation of "cDNA-selection protein fusion." Withdrawal of this rejection is therefore requested.

Rejection under 35 U.S.C. §102

Claims 1, 4-6 and 15 were rejected under 35 U.S.C. §102(b) as being anticipated by Stahl et al (Gene, 71:147-156, 1988). This rejection is traversed to the extent that it may apply to the present claims.

While not conceding the propriety of this rejection, claims 1 and 4-6 have been cancelled and claim 15 has been amended in order to expedite prosecution of the subject application. As amended, claim 15 recites that the vector comprises both a prokaryotic promoter and a eukaryotic promoter. Stahl et al does not describe such a vector. Accordingly, withdrawal of this rejection is believed to be in order.

Rejection under 35 U.S.C. §103

Claims 1-17 were rejected under 35 U.S.C. §102(a) as being unpatentable over Stahl et al, Moore et al (Analytical Biochemistry, 247:203-209, 1997) and Tashiro et al (Science, 261, 600-603, 1993). This rejection is also traversed to the extent that it may apply to the present claims.

By the present invention, fast and effective methods are provided for selecting cDNAs encoding proteins having signal sequences. Through the claimed methods a single construct can be used for initial selection of cDNAs in a prokaryotic selection system (e.g., bacterial system), which can then be followed by direct verification in a eukaryotic system.

For example, independent claim 9, as amended, reads as follows:

> 9. A method of identifying a cDNA encoding a signal sequence, comprising:
>
> directionally introducing a cDNA into a vector, said vector comprising;
>
> a prokaryotic promoter, a eukaryotic promoter, a multiple cloning site, and a nucleic acid encoding a leaderless secretable selection protein,
>
> wherein introduction of the cDNA into the multiple cloning site results in the formation of a cDNA-selection protein fusion protein-encoding nucleic acid operably linked to the prokaryotic promoter and to the eukaryotic promoter;
>
> introducing the vector comprising the fusion nucleic acid into a bacterial cell;
>
> exposing the bacterial cell containing the cDNA to a selection medium;
>
> determining growth of the bacterial cell in said selection medium, wherein growth of the bacterial cells in said selection medium is indicative of a signal sequence encoded in said cDNA;
>
> introducing the vector identified as comprising a cDNA encoding a signal sequence into a eukaryotic cell;
>
> culturing the transfected eukaryotic cell; and
>
> detecting secretion of the cDNA-selection protein fusion protein in the cell culture;
>
> wherein detection of secreted cDNA-selection protein fusion protein indicates the cDNA in the vector encodes a signal sequence.

Similarly, independent claim 10 recites, *inter alia*, use of "a fusion nucleic acid encoding a cDNA-β-lactamase fusion protein operably linked to the prokaryotic promoter and to the eukaryotic promoter." In addition, independent claim 15 recites, *inter alia*, "directionally introducing each of a plurality of cDNAs into a vector, said vector comprising a prokaryotic promoter, a eukaryotic promoter, and a nucleic acid encoding a leaderless secretable selection

protein, said introducing providing for production of a cDNA-selection protein fusion protein in a bacterial cell and in eukaryotic cell."

Thus, the claimed methods provide quick , inexpensive, and highly efficient screens for secreted and/or transmembrane proteins, which reduces the number of cDNAs that need to be screened in a eukaryotic cell of interest. The initial screen enriches for cDNAs that encode a signal sequence, which enriched cDNAs can then be screened – *using the same construct* – in a eukaryotic cell of interest. The claimed methods thus reduce the number of cDNAs that must be screened in a eukaryotic system, which screening is cumbersome, expensive, and time consuming.

These advantages are illustrated by Example 2 on page 22 of the Specification. The example shows that of the transformed bacterial clones that were selected for analysis, 50-60% of the clones confirmed the presence of a signal peptide. Subsequent validation of the secreted protiens via transfection into eukaryotic cells indicated that at least half of the clones identified in the initial bacterial screen encoded cDNA inserts that provided for secretion of the fusion protein in eukaryotic cells.

The applied art would not have guided one to the claimed subject matter. Stahl et la is directed to a method of screening signal sequence mutations that result in enhancec production of secreted proteins. Stahl et al discloses introducing a mutated signal sequence into a vector encoding a prokaryotic promoter and a leaderless β-lactamase and screening the clones in bacteria for enhanced secretion of the β-lactamase. Moore et al, on the other hand, discloses that β-lactamase can serve as a reporter gene in mammalian cells by expression from a CMV promoter, and further discloses a vector having a leaderless β-lactamase operably linked to a CMV promoter. Moore et al fails to teach or suggest use of such a vector for expression of a cDNA-β-lactamase fusion protein in a prokaryotic host cell. Finally, Tashiro et al discloses a screening method in which cDNA may be directionally cloned into a vector that consists of a eukaryotic promoter and leaderless secretable reporter protein and assaying for secreted proteins in eukaryotic cells.

However, none of the applied art, either alone or in combination, teach or suggest the use of a vector having leaderless selection protein (such as β-lactamase) operably linked to <u>both</u> a prokaryotic promoter and to a eukaryotic promoter. Moreover, none of the applied documents

suggests the claimed method which provides for identification of a cDNA encoding a signal sequence by performing an initial screen in a prokaryotic system and then screening in a eukaryotic system, where the screening uses a single construct comprising both a eukaryotic and a prokaryotic promoter.

In addition, none of the cited documents provides any motivation or suggestion that cDNAs can be screened for their ability to act as signal sequences that function in a eukaryotic cell by performing the screen first in a prokaryotic cell. There is simply no motivation provided by the documents or in the art to perform such an initial screen. Hence, the cited art also fails to provide any motivation or suggestion to use a construct that has a promoter for expression of the cDNA-selection protein fusion in both a prokaryotic cell and a eukaryotic cell. In contrast, all pending claims require use of constructs having both prokaryotic and eukaryotic promoters. Furthermore, there is no teaching or suggestion that one would reasonably expect that such an initial prokaryotic-based screen would be successful in identifying signal sequence-encoding cDNAs that function in a eukaryotic cell.

For at least the above reasons, withdrawal of the §103 rejection is believed to be in order.

CONCLUSION

In light of the above amendments and remarks, Applicants submit that the present application is fully in condition for allowance, and request that the Examiner withdraw the outstanding rejections. Early notice to that effect is earnestly solicited.

If the Examiner contemplates other action, or if a telephone conference would expedite allowance of the claims, Applicants invite the Examiner to contact the undersigned at the number listed below.

Please charge Deposit Account No. **09-0108** in the amount of $420.00 as set forth in the enclosed fee transmittal letter. If the USPTO determines that an additional fee is necessary, please charge any required fee to Deposit Account No. 09-0108.

Respectfully submitted,

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